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(54) Titre : AGENT THERAPEUTIQUE COMPORTANT DE LA BOTULINUM-NEUROTOXINE DE BOTULINUM  
(54) Title: THERAPEUTIC AGENT COMPRISING A BOTULINUM NEUROTOXIN

(57) Abrégé/Abstract:

A pharmaceutical preparation comprising one of the botulinum neurotoxins from *Clostridium botulinum* of types A, B, C, D, E, F or G or a mixture of two or more of these neurotoxins, wherein the neurotoxin or the mixture of neurotoxins is free of the complexing proteins which naturally form the botulinum neurotoxin complexes together with the neurotoxins.

**Abstract**

A pharmaceutical preparation comprising one of the botulinum neurotoxins from *Clostridium botulinum* of types A, B, C, D, E, F or G or a mixture of two or more of these neurotoxins, wherein the neurotoxin or the mixture of neurotoxins is free of the complexing proteins which naturally form the botulinum neurotoxin complexes together with the neurotoxins.

## THERAPEUTIC AGENT COMPRISING A BOTULINUM NEUROTOXIN

The present invention relates to pharmaceutical preparations which comprise a botulinum neurotoxin from *Clostridium botulinum*, the neurotoxin being free of the complexing proteins naturally present in the complex. The direct consequence thereof is the realization, on which the present invention is based, that with the free neurotoxin, in contrast to the complex, there is only a distinctly reduced, or no, induction of neutralizing antibodies in the patient. The present invention further relates to the use of botulinum neurotoxins from *Clostridium botulinum* for producing a medicine for treating disorders of the nervous system. Another aspect of the present invention relates to the use of the botulinum neurotoxins from *Clostridium botulinum* for cosmetic treatment.

*Clostridium botulinum* toxin complex type A ( $M_r$  900,000) has been employed for several years for the therapy of various dystonias. At present two different products comprising this complex are approved for the treatment of blepharospasm, hemifacial spasms and spasmodic torticollis: BOTOX® and DYSPORT®. Clinical trials of the therapy of other disorders of the nervous system (e.g. spasticities, migraine, low back pain, cervical spine

disorders, hypersalivation) are currently in progress. The products are also employed for cosmetic indications such as hyperhidrosis and pronounced wrinkling. The other *Clostridium botulinum* toxin complexes (of types B, C, D, E, F, G) are also suitable for these therapies. However, at present there is no approved product comprising one of the type B-G toxins on the market.

Botulinum toxin complexes are composed of a mixture of clostridial proteins. These are hemagglutinins with different molecular masses, a nontoxic, non-hemagglutinating protein ( $M_r$  about 120,000) and a neurotoxin ( $M_r$  about 150,000). They form an acid-stable complex which is responsible for the oral toxicity in cases of food poisoning. In contrast to the pure neurotoxin, the complex resists the aggressive environment in the gastrointestinal tract and makes enteral absorption of the neurotoxin possible, and this reaches the target cells via the bloodstream or the lymphatic system and there induces blockade of transmitter release. This is followed by a paralysis of striped and smooth muscles and cessation of various autonomic functions. Poisoned patients die of respiratory muscle failure. Since the pure neurotoxin is degraded in the gastrointestinal tract and thus does not undergo enteral absorption, it is not toxic after ingestion. On parenteral administration, the therapeutic effects of the neurotoxin and of the complex do not differ since the complex decomposes into its constituents in tissue,

and only the neurotoxin is taken up by the target cells.

For therapeutic use, the complex is in the current state of the art injected directly into dystonic or spastic muscles, where the neurotoxin is released at physiological pH from the complex and elicits the desired pharmacological effect. Although the complex is administered only in extremely low doses (1-25 ng, depending on indication and size of the affected muscle), repeated injection is followed in a considerable number of patients by formation of specific neutralizing antibodies which are also directed against the neurotoxin. The direct consequence is that antibody-positive patients no longer respond to the complex. However, they might be treated with other toxin types, although none of them is approved for therapy. When the patient has been tested with all the toxin types and has formed antibodies against them, further administration of a botulinum toxin complex (irrespective of the type) no longer provides a remedy. It must be taken into account in this connection that each dose of complex contributes to increasing the antibody titer until further administration of the complex no longer makes sense because no effect is now achieved. It often takes years for the antibody titer to fall significantly, so that these patients are not (cannot be) treated (with botulinum neurotoxin) for long periods.

The formation of specific antibodies is favored by two factors. On the one hand, the neurotoxin, fixed in the

complex, remains in the tissue for a long period and may activate immune cells which migrate into the tissue to form antibodies. The long residence time does not result in increased uptake by the target cells, however, since poisoned target cells are no longer able to take up toxin. The neurotoxin which slowly dissociates out of the complex thus now has only immunological activity. On the other hand, the proteins present in the complex intensify an immune response. Hemagglutinins are lectins, that is to say proteins which are distinguished by a high affinity for certain sugars. Because of their binding to sugar structures, lectins have immunostimulating effects. Thus, it has been possible to show that the lectins concanavalin A, phytohemagglutinin and pokeweed mitogen activate T and B lymphocytes. The hemagglutinins of the botulinum toxin complexes, which likewise bind to membrane-associated sugars, are thus able in a similar way to act as immunoadjuvants and contribute to antibody formation and thus to failure of the therapy.

The object of the inventors of the present invention was therefore to develop an alternative mode of treatment of the above-mentioned disorders and disturbances. In particular, the inventors wish to propose a suitable active ingredient with which patients who have already formed neutralizing antibodies can be treated.

To achieve the object stated above, as alternative to the two commercial type A botulinum toxin complex products,

BOTOX® and DYSPORT®, and also as alternative to the complexes described in the prior art of the other types (B, C, D, E, F, G), a novel pharmaceutical has been developed which comprises only pure neurotoxin (type A or B, C, D, E, F, G) and is free of hemagglutinins and other exogenous proteins. Because of its lower molecular mass, it diffuses more quickly to the target cells in which it is taken up, before immune cells, attracted by hemagglutinins, are activated. We found in antigenicity studies that the pure neurotoxin of all types - in distinction from commercial products of type A and the complexes of types B to G - induces no, or at the most very little, formation of antibodies. On therapeutic use of this newly developed pharmaceutical (pure neurotoxin of types A, B, C, D, E, F, G) there is no failure of therapy due to antibodies even after repeated administration. It has also been possible to show that the pure neurotoxins are, because of their immediate bioavailability, still suitable for the therapy of patients who have developed, after administration of a botulinum toxin complex, e.g. after treatment with BOTOX® or DYSPORT®, an antibody titer against the appropriate type (so-called secondary non-responders), that is to say are no longer amenable to further treatment with BOTOX® or DYSPORT®, because administration of the commercial toxins no longer alleviates the symptoms.

The pharmaceutical provided according to the invention is suitable as therapeutic composition in particular for

patients who exhibit an antibody titer against a botulinum toxin, in particular against that of type A. The novel pharmaceutical (pure neurotoxin or mixture of a plurality of pure neurotoxins) is particularly suitable for patients who exhibit an antibody titer not exceeding 50, preferably not exceeding 30, more preferably not exceeding 20, particularly preferably not exceeding 10, and very particularly preferably not exceeding 5, mU/ml. In this connection, 1 mU of antibody is the amount of antibody which neutralizes 10 U of toxin.

On the other hand, the novel pharmaceutical can be employed with particular advantage for patients who have never, or not for many years, been treated with botulinum neurotoxin, because their antibody titer is low or zero from the outset. The advantage of the present invention is then that the increase in the titer in these patients due to the treatment with the pure toxin according to the present invention is zero, or at the most very insignificant. In other words, the novel therapeutic composition can be administered over long periods without losing its effect.

The induction of antibodies during therapy with a *C. botulinum* toxin is thus prevented by administering a pure neurotoxin in place of the high molecular weight toxic complexes. The neurotoxin which has been completely separated from the complex proteins is immediately bioavailable and can bind directly to the nerve endings of the motor endplates.



One aspect of the present invention thus relates to a pharmaceutical preparation which comprises at least one of the botulinum neurotoxins from *Clostridium botulinum* of types A, B, C, D, E, F or G (or a mixture of two or more of these neurotoxins), all the neurotoxins being free of the complexing proteins naturally present in the complex.

In a preferred embodiment, the pharmaceutical preparation is such that the induction of neutralizing antibodies in the patient by the neurotoxin or the mixture of neurotoxins is reduced by comparison with the complexes or is zero.

A further preferred embodiment provides a pharmaceutical preparation which comprises as neurotoxin or as mixture of neurotoxins a natural neurotoxin or a mixture of natural neurotoxins.

A further preferred embodiment provides a pharmaceutical preparation which comprises as neurotoxin or as mixture of neurotoxins a recombinant neurotoxin or a mixture of recombinant neurotoxins.

Another preferred embodiment of the novel pharmaceutical preparation provides a preparation which comprises as neurotoxin the neurotoxin from *Clostridium botulinum* type A or B or as mixture of neurotoxins a mixture of the neurotoxins from *Clostridium botulinum* type A and B.

A further aspect of the present invention relates to the use of the botulinum neurotoxins from *Clostridium botulinum* of types A, B, C, D, E, F or G or of a mixture of two or more of these neurotoxins for producing a medicine for treating disorders of the nervous system and dystonias. The disorders of the nervous system and the dystonias are in a preferred embodiment spasmodic torticollis and blepharospasm, spasticities such as footdrop, hemifacial spasms, migraine, low back pain, cervical spine disorders or hypersalivation.

Another aspect of the present invention in turn relates to the use of the botulinum neurotoxins from *Clostridium botulinum* of types A, B, C, D, E, F or G or of a mixture of two or more of these neurotoxins for cosmetic treatment, particular preference being given to a cosmetic treatment for treating hyperhidrosis and wrinkling, especially in the facial region.

Very particularly preferred for the purpose of the present invention is the use of one of the neurotoxins alone or in a mixture for producing a medicine for treating the above-mentioned nervous disorders in persons (preferably humans, but also animals) who already exhibit neutralizing antibodies against a botulinum neurotoxin complex, in particular against the complex of *Clostridium botulinum* type A or B, or against a plurality of complexes, in particular against the complexes of *Clostridium botulinum* type A and B (so-called secondary non-responders).

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The neurotoxins, mixtures thereof and the novel pharmaceutical preparation can be in the form of an aqueous solution, in particular as aqueous solution for injection, but also as lyophilized products.

5           The pure neurotoxins of types A-G, which are known per se, were produced by the protocols present in the publications detailed in the list of references. The purification of two neurotoxins (type A and B) is described by way of example in the following examples.

10       Example 1:       Isolation of the pure neurotoxin

          The pure neurotoxin from *Clostridium botulinum* type A is obtained by a process based on the process of DasGupta & Sathyamoorthy. *Clostridium botulinum* type A is cultivated in a 20 l fermenter in a medium consisting of 2% proteose peptone, 1% yeast extract, 1% glucose and 0.05% sodium thioglycolate. After growth for 72 hours, the toxin is precipitated by adding 3 N H<sub>2</sub>SO<sub>4</sub> (final pH = 3.5). The precipitated and centrifuged biomass is extracted with a 0.2 M sodium phosphate buffer pH 6.0.

20           After removal of the nucleic acids by precipitation with protamine sulfate, the toxin is precipitated by adding ammonium sulfate. The precipitate which has been solubilized and dialyzed against 50 mM sodium phosphate pH 6.0 is bound to a DEAE-Sephadex<sup>TM</sup> column at the same pH and detached with 150 mM NaCl. This is followed by a chromatography on a QAE-Sephadex<sup>TM</sup> column which has been equilibrated with a 50 mM tris/HCl buffer pH 7.9. The toxin is eluted via an NaCl gradient. In the last step, the toxin is chromatographed on SP-Sephadex<sup>TM</sup> at pH 7.0. In this case, the bound toxin is detached from the column using an NaCl gradient (0-300 mM). The purified toxin is analyzed in an SDS polyacrylamide gel electrophoresis (SDS-PAGE) and exhibits a purity of 95 ± 5%. The biological activity is determined in the mouse LD<sub>50</sub> assay: one LD<sub>50</sub> unit corresponds to 4.8 pb of protein.

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Example 2: Production of a finished pharmaceutical containing bitulinum neurotoxin.

The purified neurotoxin from Example 1 is used to prepare a solution which comprises 200 mouse LD<sub>50</sub> units, 10 mg of sucrose and 2 mg of human serum albumin per ml. The solution (0.5 ml) is dispensed into vials and freeze-dried. The lyophilizates are reconstituted with physiological saline, and the biological activity is determined. The vials comprise 100 ± 30 LD<sub>50</sub> units.

Example 3: Isolation of pure neurotoxin B

*Clostridium botulinum* type B is cultivated in the same medium and under the same conditions as type A and is processed as far as the ammonium sulfate precipitation. This is again followed by a DEAE-Sephadex™ chromatography at pH 6.0. The fractions eluted from the column with 150 mM NaCl are combined and dialyzed against sodium phosphate pH 7.0, followed by a chromatography on QAE-Sephadex™. The toxin-containing fractions are chromatographed further on a

DEAE-Sephadex column at pH 8.5 (50 mM tris/HCl pH 8.5).

Finally, the high-purity botulinum toxin type B is obtained by a chromatography on hydroxyapatite equilibrated with 10 mM Na phosphate pH 8.0. The bound homogeneous toxin is eluted with 80 mM Na phosphate pH 8.0 and subsequently the biological activity is determined in the LD<sub>50</sub> assay ( $2-4 \times 10^7$  LD<sub>50</sub> units/mg of protein).

**Example 4:** Detection of antibodies

20 rabbits received intracutaneous injections of 25 U of BOTOX® at intervals of 14 days over a period of 12 weeks (5 injections). Serum was obtained after 3 weeks and then at intervals of 14 days.

Antibodies against *Clostridium botulinum* neurotoxin A were detected in an enzyme immunoassay by immobilizing the homogeneous neurotoxin on a microtiter plate. Antibodies binding to the neurotoxin were quantified using a second, enzyme-labeled antibody.

The result is shown in Table 1. Antibodies were detected in 5 rabbits as little as 5 weeks after the first administration. After 11 weeks, sera from 17 rabbits, that is to say 85% of the animals employed, contained antibodies against the neurotoxin. It was shown in the biological activity assay that 12 of the 17 sera contained neutralizing antibodies (Table 2).

Tab. 1 Determination of serum samples (diluted 1:100) from rabbits treated with BOTOX® using an enzyme immunoassay. OD<sub>490 nm</sub> > 0.1 are indicated. All OD values are corrected for the OD values of the preimmune sera (OD about 0.150).

Rabbit No.	3rd week	5th week	7th week	9th week	11th week
1	-	-	-	0.11	0.36
2	-	-	-	2.36	2.23
3	-	-	0.57	1.43	1.44
4	-	-	0.68	1.68	0.93
5	-	0.97	3.52	3.49	3.44
6	-	-	1.34	2.32	2.70
7	-	-	2.13	3.09	3.00
8*	-	0.53	1.47	2.75	2.75
9	-	-	0.43	2.44	2.85
10	-	-	2.99	3.15	2.73
11	-	0.10	2.42	2.45	1.93
12	-	-	-	1.13	1.95
13	-	-	-	-	1.89
14	-	-	-	-	-
15	-	-	-	-	-
16	-	-	-	-	-
17	-	2.93	3.62	3.72	3.44
18	-	-	1.18	2.28	2.62
19	-	-	0.43	0.43	0.81
20	-	1.65	3.20	2.97	2.88

\* The values were not corrected because no preimmune serum was available

"-" means optical density (OD<sub>490</sub>) < 0.1

Tab. 2 Neutralization by sera from rabbits treated with BOTOX® (week 11 after the first immunization) in the mouse hemidiaphragm assay (detection limit: 0.35 mU/ml antibodies)

Rabbits	Neutralization mU/ml
1	2.0
2	n.d.
3	n.d.
4	> 10
5	> 100
6	n.d.
7	> 10
8	> 10
9	n.d.
10	n.d.
11	n.d.
12	> 10
13	n.d.
14	n.d.
15	< 0.35
16	0.4
17	> 10
18	> 10
19	2.0
20	> 10

n.d. = not determined

**Example 5:** Antigenicity assay with market product and pure neurotoxin

After it had been shown that the complex of neurotoxin and hemagglutinins and the nontoxic, non-hemagglutinating protein induces the formation of neutralizing antibodies, the immunogenic effect of the pure neurotoxin (type A) was tested. For this purpose, 8 rabbits were treated with the toxin complex and 12 rabbits were treated with the pure toxin. 25 U of the respective product were administered intracutaneously by the method described above (see Example 1). The amount of neurotoxin, measured by weight, was the same in both products (200 pg/dose), as was demonstrated in an ELISA. BOTOX® additionally contained complex proteins (about 800 pg/dose).

Four of the eight animals treated with BOTOX® showed an antibody titer in the ELISA, whereas no antibodies against the pure neurotoxin were detectable in the 12 animals treated with pure neurotoxin. The result was confirmed in the biological activity assay. All four rabbit sera contained neutralizing antibody titers preventing an effect of the toxin (Table 3).



Tab. 3 Neutralization by sera (diluted 1:3) from rabbits treated with BOTOX® (week 11 after the first immunization) in the mouse hemidiaphragm assay (detection limit: 1 mU/ml antibodies)

Rabbits	Neutralization mU/ml
1	12 mU
2	> 30 mU
3	4.5 mU
8	> 30 mU

**Example 6:** (Comparative Example)

This experiment compared the antibody formation due to BOTOX® with that due to DYSPORT®. For this purpose, groups of ten rabbits were treated either with BOTOX® (group 1), with DYSPORT® (group 2) or with the pure neurotoxin (group 3) in accordance with the scheme described.

Whereas more than 50% of the animals formed a neutralizing antibody titer in group 1 and 2, the sera from the animals in group 3 were free of antibodies.

**Example 7:** Clinical test

A patient (45 years of age) who had been treated for a period of 5 years with BOTOX® for spasmodic torticollis had developed an antibody titer of 3 mU/ml of serum. Neither BOTOX® nor DYSPORT® was therapeutically effective for this patient. An attempt at therapy with the pure botulinum

neurotoxin in a dose of 145 U, which was equivalent to the last BOTOX® dose injected, resulted within 72 hours in loosening of the muscle, normalization of the posture of the head and disappearance of the muscle pain. No adverse effects occurred.

**Example 8: Clinical test**

A patient (52 years of age) had been treated with BOTOX® for 3 years for cerebral palsy. He had developed an antibody titer of 1 mU/ml of serum and it was thus necessary to discontinue the therapy. Injection of 200 U of pure neurotoxin made successful therapy possible.

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## CLAIMS:

1. Use of a botulinum neurotoxin from Clostridium botulinum of type A, B, C, D, E, F or G or a mixture of two or more of said neurotoxins, wherein said neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, for the manufacture of a pharmaceutical for cosmetic treatment or treatment of disorders of the nervous system or dystonia selected from migraine, low back pain, hypersalivation, spasmodic torticollis, blepharospasm, spasticities, hemifacial spasms and neuromuscular disorders of the cervical spine, in a human or animal subject already exhibiting neutralizing antibodies against botulinum neurotoxin complexes.

2. The use according to claim 1 wherein said subject exhibits neutralizing antibodies against either or both of Clostridium botulinum neurotoxin complex type A and Clostridium botulinum neurotoxin complex type B.

3. The use according to claim 1 or claim 2 wherein said pharmaceutical is for cosmetic treatment.

4. The use according to claim 3, wherein said cosmetic treatment is in respect of hyperhidrosis.

5. The use according to claim 3, wherein said cosmetic treatment is in respect of wrinkling.

6. The use according to claim 3, wherein said cosmetic treatment is in respect of facial wrinkling.

7. The use according to claim 1 or 2, wherein said pharmaceutical is for treatment of disorders of the nervous system or dystonia selected from migraine, low back pain, hypersalivation, spasmodic torticollis,

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blepharospasm, spasticities, hemifacial spasms and neuromuscular disorders of the cervical spine.

8. The use according to claim 7, wherein said disorder or dystonia is migraine.

5 9. The use according to claim 7, wherein said disorder or dystonia is low back pain.

10. The use according to claim 7, wherein said disorder or dystonia is hypersalivation.

10 11. The use according to claim 7, wherein said disorder or dystonia is spasmodic torticollis.

12. The use according to claim 7, wherein said disorder or dystonia is blepharospasm.

13. The use according to claim 7, wherein said disorder or dystonia is spasticity.

15 14. The use according to claim 7, wherein said disorder or dystonia is hemifacial spasm.

15. The use according to claim 7, wherein said disorder or dystonia is a neuromuscular disorder of the cervical spine.

20 16. The use according to any one of claims 1 to 15, comprising use of a botulinum neurotoxin from Clostridium botulinum of type A.

25 17. The use according to any one of claims 1 to 16, comprising use of a botulinum neurotoxin from Clostridium botulinum of type B.

18. The use according to any one of claims 1 to 17, comprising use of a botulinum neurotoxin from Clostridium botulinum of type C.

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19. The use according to any one of claims 1 to 18, comprising use of a botulinum neurotoxin from Clostridium botulinum of type D.

20. The use according to any one of claims 1 to 19, comprising use of a botulinum neurotoxin from Clostridium botulinum of type E.

21. The use according to any one of claims 1 to 20, comprising use of a botulinum neurotoxin from Clostridium botulinum of type F.

22. The use according to any one of claims 1 to 21, comprising use of a botulinum neurotoxin from Clostridium botulinum of type G.

23. Use of a botulinum neurotoxin from Clostridium botulinum of type A, B, C, D, E, F or G or a mixture of two or more of said neurotoxins, wherein said neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, for cosmetic treatment or for treatment of disorders of the nervous system or dystonia selected from migraine, low back pain, hypersalivation, spasmodic torticollis, blepharospasm, spasticities, hemifacial spasms and neuromuscular disorders of the cervical spine, in a human or animal subject already exhibiting neutralizing antibodies against botulinum neurotoxin complexes.

24. The use according to claim 23 wherein said subject exhibits neutralizing antibodies against either or both of Clostridium botulinum neurotoxin complex type A and Clostridium botulinum neurotoxin complex type B.

25. The use according to claim 23 or claim 24 wherein said use is for cosmetic treatment.

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26. The use according to claim 25, wherein said cosmetic treatment is in respect of hyperhidrosis.

27. The use according to claim 25, wherein said cosmetic treatment is in respect of wrinkling.

5 28. The use according to claim 25, wherein said cosmetic treatment is in respect of facial wrinkling.

29. The use according to claim 23 or 24, wherein said use is for treatment of disorders of the nervous system or dystonia selected from migraine, low back pain, 10 hypersalivation, spasmodic torticollis, blepharospasm, spasticities, hemifacial spasms and neuromuscular disorders of the cervical spine.

30. The use according to claim 29, wherein said disorder or dystonia is migraine.

15 31. The use according to claim 29, wherein said disorder or dystonia is low back pain.

32. The use according to claim 29, wherein said disorder or dystonia is hypersalivation.

20 33. The use according to claim 29, wherein said disorder or dystonia is spasmodic torticollis.

34. The use according to claim 29, wherein said disorder or dystonia is blepharospasm.

35. The use according to claim 29, wherein said disorder or dystonia is spasticity.

25 36. The use according to claim 29, wherein said disorder or dystonia is hemifacial spasm.

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37. The use according to claim 29, wherein said disorder or dystonia is a neuromuscular disorder of the cervical spine.

38. The use according to any one of claims 23 to 37, comprising use of a botulinum neurotoxin from Clostridium botulinum of type A.

39. The use according to any one of claims 23 to 38, comprising use of a botulinum neurotoxin from Clostridium botulinum of type B.

40. The use according to any one of claims 23 to 39, comprising use of a botulinum neurotoxin from Clostridium botulinum of type C.

41. The use according to any one of claims 23 to 40, comprising use of a botulinum neurotoxin from Clostridium botulinum of type D.

42. The use according to any one of claims 23 to 41, comprising use of a botulinum neurotoxin from Clostridium botulinum of type E.

43. The use according to any one of claims 23 to 42, comprising use of a botulinum neurotoxin from Clostridium botulinum of type F.

44. The use according to any one of claims 23 to 43, comprising use of a botulinum neurotoxin from Clostridium botulinum of type G.

45. A pharmaceutical preparation comprising a pharmaceutically acceptable carrier and a botulinum neurotoxin from Clostridium botulinum of type A, B, C, D, E, F or G or a mixture of two or more of said neurotoxins, wherein said neurotoxin or mixture of neurotoxins is free of the complexing proteins which



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naturally form complexes with botulinum neurotoxins, for cosmetic treatment or for treatment of disorders of the nervous system or dystonia selected from migraine, low back pain, hypersalivation, spasmodic torticollis, blepharospasm, spasticities, hemifacial spasms and neuromuscular disorders of the cervical spine, in a human or animal subject already exhibiting neutralizing antibodies against botulinum neurotoxin complexes.

46. The pharmaceutical preparation according to claim 45 wherein said subject exhibits neutralizing antibodies against either or both of Clostridium botulinum neurotoxin complex type A and Clostridium botulinum neurotoxin complex type B.

47. The pharmaceutical preparation according to claim 45 or claim 46 wherein said use is for cosmetic treatment.

48. The pharmaceutical preparation according to claim 47, wherein said cosmetic treatment is in respect of hyperhidrosis.

49. The pharmaceutical preparation according to claim 47, wherein said cosmetic treatment is in respect of wrinkling.

50. The pharmaceutical preparation according to claim 47, wherein said cosmetic treatment is in respect of facial wrinkling.

51. The pharmaceutical preparation according to claim 45 or claim 46, wherein said use is for treatment of disorders of the nervous system or dystonia selected from migraine, low back pain, hypersalivation, spasmodic torticollis, blepharospasm, spasticities, hemifacial spasms and neuromuscular disorders of the cervical spine.

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52. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is migraine.

53. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is low back pain.

54. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is hypersalivation.

55. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is spasmodic torticollis.

56. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is blepharospasm.

57. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is spasticity.

58. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is hemifacial spasm.

59. The pharmaceutical preparation according to claim 51, wherein said disorder or dystonia is a neuromuscular disorder of the cervical spine.

60. The pharmaceutical preparation according to any one of claims 45 to 59, comprising use of a botulinum neurotoxin from *Clostridium botulinum* of type A.

61. The pharmaceutical preparation according to any one of claims 45 to 60, comprising use of a botulinum neurotoxin from *Clostridium botulinum* of type B.

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62. The pharmaceutical preparation according to any one of claims 45 to 61, comprising use of a botulinum neurotoxin from Clostridium botulinum of type C.

5 63. The pharmaceutical preparation according to any one of claims 45 to 62, comprising use of a botulinum neurotoxin from Clostridium botulinum of type D.

64. The pharmaceutical preparation according to any one of claims 45 to 63, comprising use of a botulinum neurotoxin from Clostridium botulinum of type E.

10 65. The pharmaceutical preparation according to any one of claims 45 to 64, comprising use of a botulinum neurotoxin from Clostridium botulinum of type F.

15 66. The pharmaceutical preparation according to any one of claims 45 to 65, comprising use of a botulinum neurotoxin from Clostridium botulinum of type G.